Mammot user manual (based on version v1.11).

Ed Ryder, January 2006

Chip administration:

Adding a new tiling path chip:

1. Choose the species and genome release the tiling path was designed on using the drop down menus (steps 1 and 2).
2. For step 3 we have to insert the data for the tiling path, as well as give it a name and some sundry information. For ease of use data can be directly pasted into the text box labelled 'chip information'. If you designed your chip using the tiling perl scripts included in the package simply pasting in the contents of the *project_chip_import.out* file will work fine. If however the data came from a different source the following tab-delimited format must be adhered to for it to work:

Field	**Format(max)**
Tile name	varchar(64)
Chromosome	varchar(8)
Start location	int(10)
Stop location	int(10)
5' primer sequence	varchar(128)
3' primer sequence	varchar(128)
Whole tile sequence	text
notes	text
5' primer Tm	float
5' primer CG%	float
3' primer Tm	float
5' primer CG%	float
% repeat sequence	int(3)
%CG	float
Tile size bp	int(6)

If information for particular fields is not available, please enter \N instead (this tells the database that this field has no value). You may find that some of the functions of the viewer will not work properly however (eg no sequence will be available to view if you haven't entered any).

As an example, the file *dros1_chip_import.out* available from the web site contains the tiling pathway used in the demonstration site.

Adding oligonucleotide-based tiling arrays:

Although originally designed for PCR tiles, Mammot is capable of displaying information on oligonucleotide-based arrays too (and probably lots of other types – if it has a start, stop and chromosome location it can display it,

although certain other options may not available). Below is an example of how you might construct the tab-delimited file for one.

Field	Value
Tile name	Dros1_oligo1
Chromosome	2L
Start location	3520538
Stop location	3520561
5' primer sequence	GCTTTATGAGCTTCACAGGAGTT
3' primer sequence	\N
Whole tile sequence	GCTTTATGAGCTTCACAGGAGTT
notes	\N
5' primer Tm	59.10
5' primer CG%	43.48
3' primer Tm	\N
5' primer CG%	\N
% repeat sequence	0
%CG	43.48
Tile size bp	23

The file *dros1_oligo_import.out* available from the web site contains a brief example of an oligo array suitable for importing.

3. Clicking on 'add data to database' should upload the data into the database.

Adding a new tiling path chip:

It may be in the future that you wish to add more tiling paths to your chip or simply extend an existing one.

1. Choose the chip you wish to append from the menu.
2. Paste in any new information using the data format outlined for adding a new chip.
3. Click on 'add data to database' to upload the new information.

Deleting a tiling path chip:

If you are finished using a chip you can delete it to clear some space in the database. **WARNING!! DELETING A CHIP IS IRREVERSIBLE** so only perform this action if you really want to delete it.

1. Choose the chip you wish to delete and click on 'delete'.
2. You will now get one last opportunity to change your mind!

Adding PCR results:

It may be useful when interpreting results to have some experimental data handy about how the amplification of the tiling pathway went, as PCR is isn't always 100% successful and /or specific.

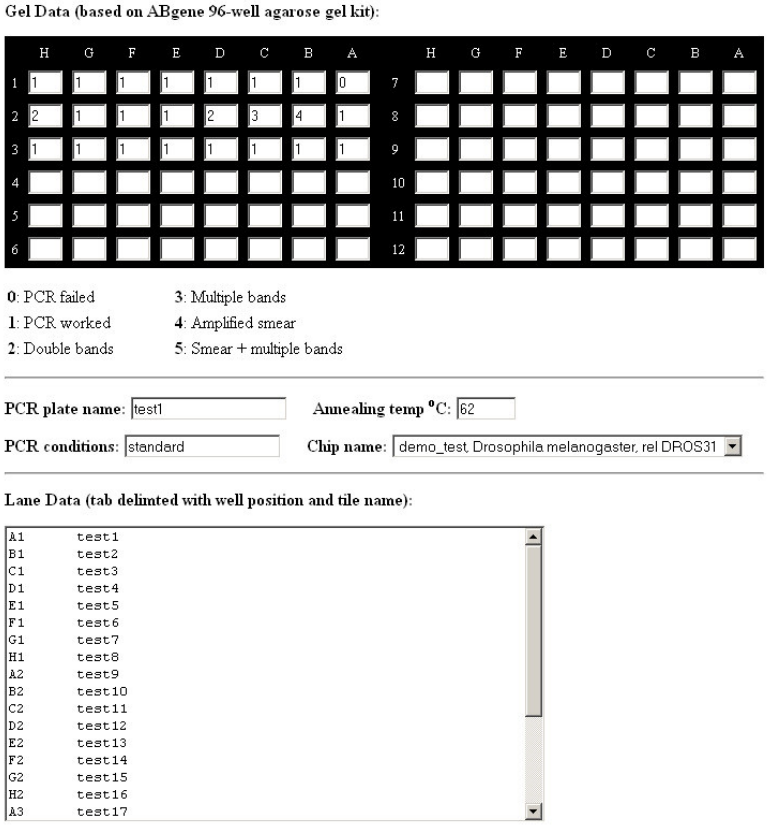
PCR results are entered using a simple numbered code into the diagram of the gel based on a 96-well plate (it may seem a bit odd to have the columns back to front but this is how our gel system works). The current template is based on the ABgene 96-well kit and more templates may be available on request in the future.

1. Enter the correct codes for each well in the plate.
2. Enter supplementary information about the name of the plate, PCR conditions etc.
3. Lane data can be pasted directly in from a spreadsheet into the box provided. This ties the sample and well position to the PCR result and must be in the tab-delimited format 'well_position, tile_name'.

   e.g `A1  sample1`
   `B1  sample2` etc

4. Clicking on 'add data' will upload the information to the database.

Example of PCR results.


Gel Data (based on ABgene 96-well agarose gel kit):
H G F E D C B A H G F E D C B A
1 1 1 1 1 1 1 1 0 7
2 2 1 1 1 2 3 4 1 8
3 1 1 1 1 1 1 1 1 9
4 10
5 11
6 12
0: PCR failed
1: PCR worked
2: Double bands
3: Multiple bands
4: Amplified smear
5: Smear + multiple bands
PCR plate name: test1
Annealing temp °C: 62
PCR conditions: standard
Chip name: demo_test, Drosophila melanogaster, rel DROS31
Lane Data (tab delimted with well position and tile name):
A1 test1
B1 test2
C1 test3
D1 test4
E1 test5
F1 test6
G1 test7
H1 test8
A2 test9
B2 test10
C2 test11
D2 test12
E2 test13
F2 test14
G2 test15
H2 test16
A3 test17

Adding a genome feature track:

Custom genome features can be added here to help in the analysis of any biological significance to experimental results. Custom features are associated with species and genome release rather than a particular tiling path chip and so will be available to anyone viewing any other chips using the same parameters.

1. Enter the name of the feature.
2. Paste in feature information. This must be tab-delimited and have the following format shown below.

Field	Format
Start location bp	int(11)
Stop location bp	int(11)
Chromosome	varchar(4)
Strand (1 or –1)	int(1)
Name of individual feature in track (\N for none)	varchar(64)
Origin code (1 for experimental, 2 for theoretical	int(1)

3. Click on ‘add data’ to upload the information to the database.

Deleting a genome feature track:

Deleting a feature track is irreversible so please be sure you really want to do it before proceeding.

1. Select the species and genome release of the feature you wish to delete.
2. Choose the feature you wish to delete and click on ‘delete feature’ to remove it from the database.

Adding an experiment group:

To aid in data organisation when projects begin to get large, experiments are ordered into groups. To create a new group, choose the tiling chip the group is associated with, the group name and description. This group will now be available as a menu choice when new experiments are added. Experiments not assigned a group will be placed into ‘misc’.

Delete experimental data:

You can use this option to delete any experiments. Choose the tiling chip and experiments using the menus and click on ‘delete experiments’ to continue. Deleting an experiment is irreversible so please be sure you really want to do it before proceeding.

Entering experiments:

Experimental data is entered using a simple form.

1. Choose the experiment group you wish these data to be associated with. Experiment groups are set up in the chip admin pages.
2. Enter the name of the person who performed the experiment.
3. Enter the name of the experiment.
4. The experiment description should contain details about the experiment (eg conditions, anti body used in ChIP etc). This will appear as a mouse-over popup on the main viewer.
5. Spot and value data can be pasted in directly from a spreadsheet. It must be in the tab-delimited format of 'tile name' and 'value'. Future releases will allow entry of p values and standard deviation data. An example of the correct format is shown below.

```
tile_one  1.23
tile_two  0.12
tile_three     0.23
```

6. Click on 'add data to database' to upload the data.

View results:

Use these pages for exporting data into BED format for use with the UCSC genome browser (http://genome.ucsc.edu/cgi-bin/hgGateway).

1. Choose the tiling chip you wish to use.
2. Click on the experiments you wish to export.
3. Click on 'export experiments'. This will write a file in the correct format for use in the UCSC browser and provide you with a link to that file.
4. Full instructions on what to do next are available from the UCSC site.

Chip viewer:

Select the tiling chip you wish to view and click on 'choose chip'. A table will then be displayed showing all the tile locations, primer sequences etc. For large or multiple tiling pathways this function may take a fairly long time to run. Clicking on 'sequences' or 'features' on the table will load up information about the selected tile in a separate window.

Batch tile viewer:

You can use this page to generate a batch report for tiles of interest. Paste in the tile names and scores to generate a table of genes and any GO reports associated with those tiles.

Top tiles viewer:

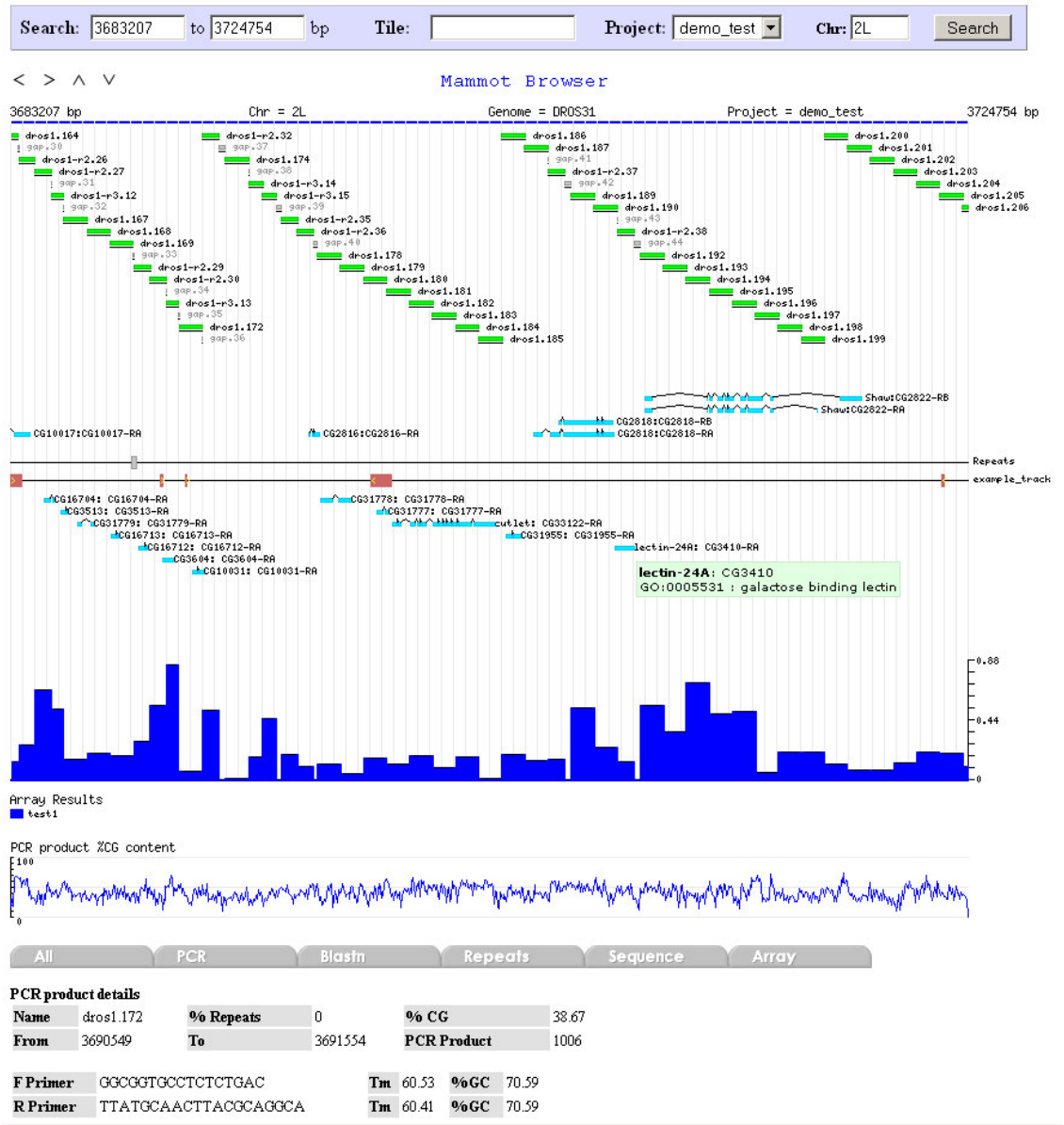
This functions in a similar manner to the ‘batch tiles’ viewer except that it generates a report from the top scoring tiles in a particular experiment. You can choose the experiment and number of tiles to generate reports from the menus provided.

Previous Uploads:

This page provides a list of BED format files previously exported, their URL and the experiments chosen for the export.

The mammot viewer:

The mammot viewer provides a visual way of representing the tiling pathway and any experimental data, along with custom chip information. The main window is shown below.



Search bar:

1. Choose the tiling chip and chromosome you wish to perform the search on.
2. Enter the start and stop chromosome locations to display. Larger ranges will take longer to display than smaller ones.
3. You can also search for a specific tile which will zoom the display to a 5kb search window.
4. Click on ‘search’ to zoom the window to the selected range.

Main pane:

The main window contains the navigation bar, tiling pathway, gene models and feature tracks.

Navigation bar:

< > ^ v

Use these arrows to navigate around the selected range: left and right will shift the range along the chromosome, and the up and down arrows will zoom the search range in and out.

Tiling path:

The tiling path is a graphical representation of the PCR products designed in the tiling phase of the project. Tiles are colour coded depending on the amount of screened repeat DNA (may not be available if the tiling perl script was not used).

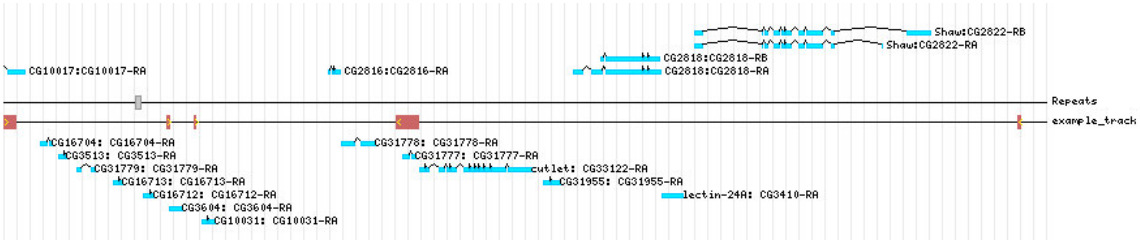
- Green: < 10% screened repeats
- Yellow: 10% to 20%
- Orange: 20% to 30%
- Red: Over 30%
- Grey: computed gap in the tiling path

The bar below the tile displays the PCR status of the tile.

- Green: tile amplified successfully
- Red: tile amplified non-specifically
- Black: tile not amplified

Clicking on a tile will display information associated with it in the Information Pane at the bottom of the browser window.

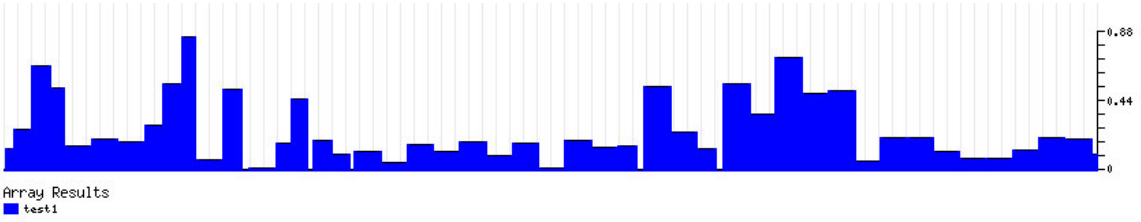
Gene model and features:



The gene model tracks show graphical representations of genes associated with the selected species and genome release. Hovering the mouse over a model will produce a popup box giving the GO report (if available) of the gene.

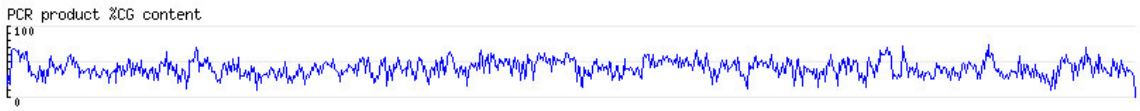
The feature tracks will show any features currently selected to display, as well as any repeat features (simple repeats are not shown as this would clutter the display too much). Features are colour coded for ease of identification and any strand information is represented by an arrow in the feature (pointing right for plus strand, left for minus strand). Hovering the mouse over a feature will produce a popup box giving further information about it (repeat type, feature name etc).

Experiment track:



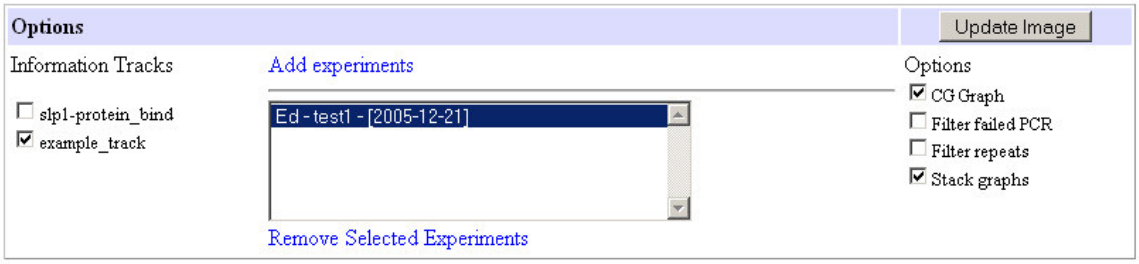
This shows the data from the experiments selected in the options, scaled against the maximum score for the experiment. Multiple experiments can be viewed on the same graph or stacked in different graphs. Hovering the mouse over the bars will show the score for that tile, and clicking on it will display the array scores for all selected experiments for that tile in the Information Pane. Placing the mouse over the coloured legend will display the experiment description.

CG plot:



If the 'CG graph' option is selected a graph will be plotted based on the %CG content of the sequence of the tiles. Selecting a region of chromosome that does not contain any tiles will result in an incorrect CG plot.

The options box:



The options box consists of three sections – the information track selector, the experiment selector and other visual options.

The 'information tracks' part shows the genomic features associated with the chosen species and genome release. Tick the boxes to select which features you would like displayed (apart from the repeat elements, which are always visible).

Click on 'update image' to apply the selections.

Adding experiments:

Click on 'add experiments' and a new window will appear listing experiments according to the defined groups associated with the selected tiling chip project. To add experiments to the viewing queue, highlight them by clicking (you can add multiple experiments by holding down the CTRL key) and click on 'select from list'. The experiments will then appear in the experiment window on the main page.

Click on 'update image' to apply the selections.

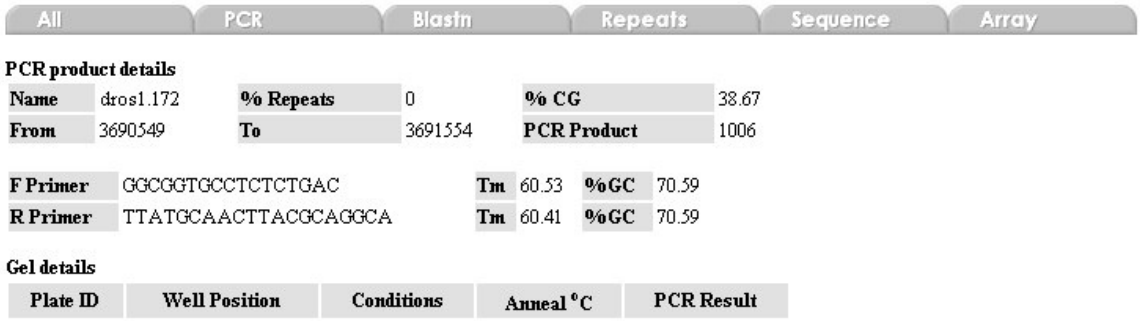
Other options:

Click on the checkboxes to activate the options. Click on 'update image' to apply the selections.

- CG graph – display %CG plot
- Filter failed PCR – Tiles in which the PCR failed will be greyed out in the experiment plot.

- Filter repeats – Tiles with a large repeat content will be greyed out in the tile plot.
- Stack graphs – Experiments will be plotted on their own graph. Un-ticking this box with mean experiments are combined on the same graph.

Information Pane:



The information pane contains information about the tiles in the pathway. Clicking on a tile in the main window brings up information about it. Click on the labelled tabs for specific information.

- All – summary of information
- PCR – Tile location, amplification details and conditions. Any gel details if available.
- Blastn – Genome alignments of the primers and products if available.
- Repeats – Repeat content details if available.
- Sequence – Tile sequence. Repeat-masked regions in lowercase are highlighted in red.
- Array – Ratio scores for any selected experiments in the session